THE EFFECTS OF HYDROGEN ION CONCENTRATION, FATTY ACIDS AND VITAMIN C ON THE GROWTH OF FUNGI[1,2]

SAMUEL M. PECK, M.D. AND HERBERT ROSENFELD, M.D.

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The interest of the authors in the fungicidal properties of the fatty acids and their salts was aroused during the investigations which are being conducted on sweat. It was observed that sweat had fungicidal properties which were probably due to a certain extent to some of the fatty acids.

It has been known for some time in certain industries that moulds can be inhibited by some of the salts of the fatty acids. It very soon became apparent that any study of this problem must take into consideration the effects on the growth of the fungi of the hydrogen-ion concentrations resulting from the addition of any of these acid compounds to the media.

Many of the problems which have arisen have as yet not been solved. Clinical investigations of the treatment of fungous disease by means of some of the fatty acids and their salts seemed to have demonstrated that they can be of use in the treatment of these conditions. This will shortly be reported in another publication.

The importance of this approach to the problem of the treatment of fungous infections does not lie in the discovery of any startlingly new fungicidal agents but in the concept that the use of some of these products approaches a more physiologic method of therapy. It is probably for this reason that this form of

[1] From the Laboratories of The Mount Sinai Hospital, and from the Skin and Cancer Unit of New York Post-Graduate Medical School and Hospital, Columbia University.

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therapy causes very little irritation and thus tends to decrease the incidence of trichophytids which very often are sequelae of more irritating methods of treatment.

Materials and Methods

The method of preparation of media is given in some detail since the growth characteristics and even the effects of hydrogen-ion concentration on the growth of fungi depends very much on the type of media used (1, 2).

Sabouraud's Media. For the preparation of the solid media a standard Sabouraud's dextrose agar dehydrated (Difco) was used. Sixty-five grams of this material was dissolved in a liter of distilled water. It was then sterilized with steam at 15 lbs. for 20 minutes. The final pH of this medium was about 5.6.

The plates, after cooling, were inoculated on the surface with *Trichophyton Gypseum* or *Epidermophyton Inguinale.* In using the plate method for Monilia, 1 cubic centimeter of a dilute suspension of spores was placed in the bottom of the plate and just before the medium began to harden it was poured over them with mixing. In judging the growth of the Trichophyton and Epidermophyton, the size of the colony at the end of two weeks was used as a standard. The fungicidal agent was added to the medium in the required concentration just before the plates were poured. Five plates were used for each concentration studied. In the case of the Monilia the number of colonies and general vigor of growth was taken as a standard.

Sabouraud's Bouillion. Twenty grams of dextrose plus 10 grams of Neopeptone (Difco) were added to 100 cc. of distilled water. The mixture was put into an Arnold autoclave for 10 minutes, filtered through ordinary filter paper while still hot, then autoclaved at 10 lbs. for 10 minutes. This medium as prepared was not very suitable for the growth of fungi as will be seen in a number of experiments that will be cited later.

If the various amounts of the substances to be tested were added to medium in the usual way, there would result differing amounts of nutrient agar plus peptone in every tube. This, too, of course, might be a factor of error in judging the final effects. This factor was corrected by adding saline to the various substances investigated to make the desired concentration, the organisms themselves being suspended in the 10 X Sabouraud's broth, and adding enough of this spore suspension to each tube to make the final concentration of medium equivalent to the ordinary Sabouraud's bouillion, that is, 1/10 of the 10 X media.

Pellicle Method. In using Sabouraud's bouillion one has a very convenient method for testing the fungicidal power of the various fatty acids and their salts on the *Trichophyton Gypseum.* It is possible to test their effect on this organism because after the spores are inoculated in the depths of the fluid media, they first grow in the form of a cloudy suspension, thus growing partly under anaerobic conditions, and finally they reach the top and form a surface pellicle. The degree of growth is measured by the amount of subsurface floculate and by the size of the surface pellicle.

As will be seen from the experiments by the plate method, the *Trichophyton Gypseum* is much more resistant to fungicides than the *Epidermophyton Inguinale* and, therefore, it can be taken for granted that a concentration of any one of the

test substances which was lethal for *Trichophyton Gypseum* would also prove to be fungicidal for the *Epidermophyton Inguinale.*

Organisms Investigated. The effects of the fungicidal activities of the various substances was tested on *Trichophyton Gypseum, Epidermophyton Inguinale* and *Monilia Albicans.*

THE EFFECT OF THE HYDROGEN ION CONCENTRATION ON THE GROWTH OF THE FUNGI

Method of Buffering

Method I. To 25 cc. of the media there were added enough normal HCl or NaOH to yield the required hydrogen-ion concentration. The final pH was at first controlled by indicator methods, using Clark's color charts as a standard. Color charts and even comparator tubes are only fairly accurate. Therefore, in all instances, the pH given in all of the tables was measured also with glass electrodes and a vacuum tube pH meter.

Method II. McIllvaine's buffers were added to the media in the proportion of 2 cc. of the required buffer concentration to 10 cc. of the media. This was again controlled for the final pH value. McIllvaine's buffers consist of citric acid and disodiumphosphate mixtures. These give a range of pH from 2.2 to 8.0. To obtain a pH of 9.0, disodiumphosphate alone was added; for the pH of 10.0, approximately 40 cc. of disodiumphosphate plus 20 drops of normal NaOH was needed. The final pH was then compared with standard Kolthoff buffers and also controlled by electrometric measurements.

Experiment I

Sabouraud's bouillion was prepared varying in pH from 3.0 to 10.0. There were three parallel series of tubes. One series consisted of the ordinary media in which the required pH was produced by adding HCl or NaOH according to Method I; to another series of tubes McIllvaine's buffers were added to obtain the pH range. The third series consisted of the 10 X media plus McIllvaine's buffers. A suspension of spores of *Trichophyton Gypseum* was inoculated in the depths of each tube.

Results. The results of this experiment are summarized in table I. It can be seen that the fungi can grow to some slight extent even at pH 3.4. When NaOH or HCl were used, good growth took place at a pH range from 4.4 to 10.0, the only difference being that at pH of 9.0 and 10.0 the subsurface growth was markedly diminished.

With McIllvaine's buffers added, the optimum growth was at a pH of 6.0; good growth at pH 5.0; some at pH 4.4 and very little to none at all at the extreme ranges of the series. When these same buffers are used in a 10 X media the retardation of growth at either end of the series was much more marked.

Experiment II

A solid Sabouraud's medium was prepared ranging in pH from 4.0 to 10.0. It was not possible to prepare this medium at pH 3.0, as it did not harden at this pH value. As in the previous experiment, the pH was obtained by both the

Methods I and II, i.e., on the one hand using NaOH and HCl and on the other McIllvaine's buffers.

It can be seen from table II that when compared with the control at the end of two weeks there was practically no difference in the size of the colony of *Trichophyton Gypseum* in the whole range from pH 4.0 to 10.0, when no buffer was present (fig. 1). There was some retardation of *Epidermophyton Inguinale* at pH 4.0 in the same series. *Monilia Albicans* grew equally well at pH 4.0 to 9.0. There was some retardation at pH 10.0.

The plates with McIllvaine's buffers also showed growth of all organisms throughout the whole pH range. However, *Trichophyton Gypseum* showed definite retardation as compared with controls and with the previous unbuffered series at all pH values except 7.0 and 8.0. *Epidermophyton Inguinale* was definitely retarded throughout the whole range of pH values as compared to the previ-

TABLE I

Effect of hydrogen ion concentration on growth of Trichophyton gypseum in Sabouraud's bouillon (14 days)

METHOD OF ADJUSTMENT OF pH	pH 3.0	pH 3.4	pH 4.0	pH 4.4	pH 5.0	pH 6.0	pH 7.0	pH 8.0	pH 9.0	pH 10.0
HCl or NaOH added	0	±	+	+++	++++	++++	++++	++++	++++*	++++†
McIllvaine's buffers added	0	±	+	++	+++	++++	+	+	±	0
10 X media plus McIllvaine's buffers	0	0	0	Not done	+	+++	+	+	±	0

0 = No growth.
± = Slight growth below surface.
+ = Moderate floc below surface.
++ = Moderate subsurface growth with a small colony floating on surface.
+++ = Partial covering of surface or as a band on side of tube with heavy subsurface floc.
++++ = Heavy growth of surface mycelium with heavy subsurface flocculate.
* Very wide pellicle on surface but moderate flocculate subsurface.
† Very wide pellicle on surface but no subsurface growth.

ous series. Optimum growth was obtained at pH 7.0. There was a slight reduction in the growth of Monilia Albicans throughout the whole range (fig. 2).

Experiment III

The question then naturally arises why there is a retardation of growth in the series containing McIllvaine's buffers. The retardation on the acid side could be due to the concentration of the citric acid, while that on the alkaline side to the disodiumphosphate.

To answer this question a series of tubes of Sabouraud's boullion were prepared with concentrations of citric acid ranging from 5 to 0.01 per cent. A parallel series of tubes in which the disodiumphosphate was used was also made. Both series were then inoculated with *Trichophyton Gypseum* as in the pellicle method.

It required a concentration of 0.3 per cent citric acid to inhibit growth completely, while a 0.01 per cent solution of this compound permitted 100 per cent growth. Since the pH of a 0.3 per cent citric acid solution was 2.9, the apparent

TABLE II

Effect of the hydrogen ion concentration on growth by the plate method

pH	TRICHOPHYTON GYPSEUM		EPIDERMOPHYTON INGUINALE		MONILIA ALBICANS	
	With buffer	No buffer	With buffer	No buffer	With buffer	No buffer
	cm.	*cm.*	*cm.*			
4.0	3.2	4.6	0.9	1.4	Slight reduction in growth	Many small colonies; some larger
5.0	2.8	4.8	1.1	2.4	Slight reduction in growth	Many small colonies; some larger
6.0	3.9	4.8 Few bacteria	1.1	2.3	Slight reduction in growth	Plate heavily seeded
7.0	4.8	4.4* Many bacteria	1.3	2.3 Many bacteria	Slight reduction in growth	Plate heavily seeded
8.0	4.6	4.5 Many bacteria	1.1	2.3 Many bacteria	Slight reduction in growth	Plate heavily seeded
9.0	4.3	5.0 Many bacteria	1.1	2.7 Bacteria and yeasts	Slight reduction in growth	Colonies larger but fewer (slight inhibition)
10.0	4.0	4.7 Some bacteria	0.9	2.7 Few bacteria	Slight reduction in growth	Colonies larger but fewer (slight inhibition)
5.8	Control	4.7		2.2		Heavily seeded

* The average diameter of colonies is given at end of two weeks. Five plates inoculated for each pH. The agar did not harden at pH of 3.0.

At pH 7 and 8 there was a definite zone of lysis for bacteria at margin of colonies both for trichophyton and epidermophyton.

fungicidal effect of citric acid can be considered to be due to the pH produced. This is further substantiated by the fact that growth in fluid media with and without buffer is about the same up to a pH of 4.0; the buffer up to that point consisting almost entirely of citric acid.

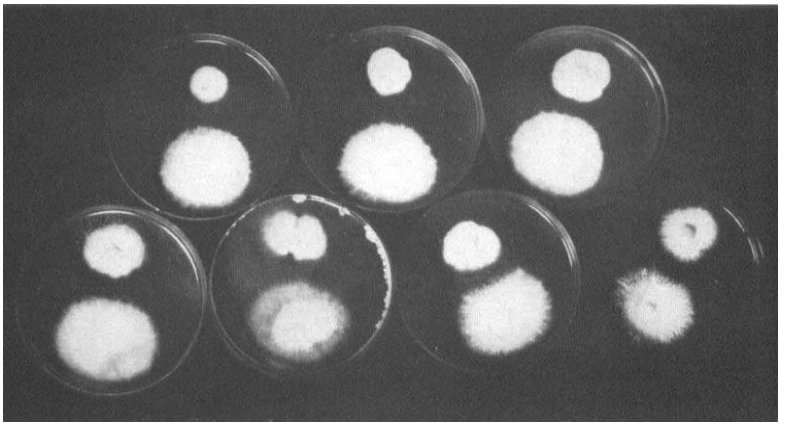


FIG. 1. GROWTH OF TRICHOPHYTON GYPSEUM (LARGE COLONY) AND EPIDERMOPHYTON INGUINALE ON SABOURAUD'S MEDIUM FROM pH 4.0 (UPPER LEFT) TO pH 10.0 (LOWER RIGHT)

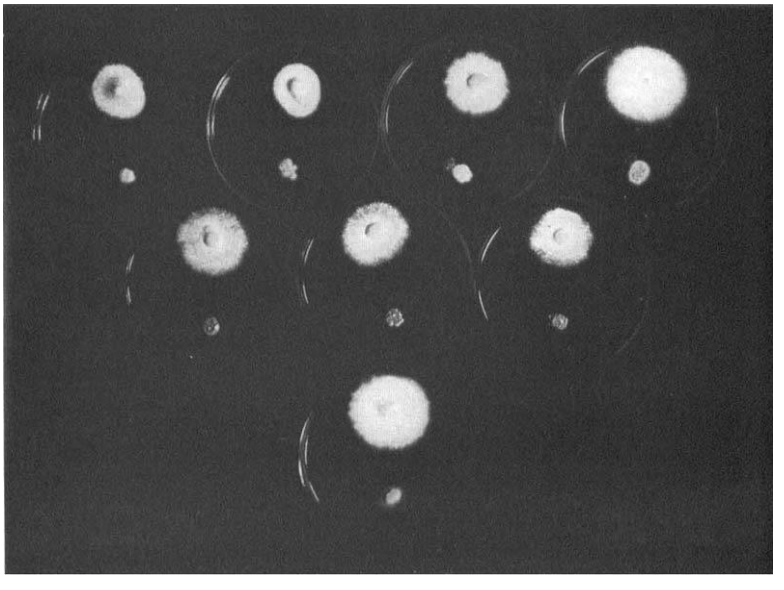


FIG. 2. GROWTH OF TRICHOPHYTON GYPSEUM AND EPIDERMOPHYTON INGUINALE ON SABOURAUD'S MEDIUM PLUS MCILLVAINE'S BUFFERS FROM pH 4.0 (UPPER LEFT) TO pH 10.0 (LOWER RIGHT)

Control (third row center)

It required a 5 per cent solution of disodiumphosphate to inhibit growth completely. The pH of that solution was 7.9. A concentration of 0.3 per cent allowed 100 per cent growth. It can be seen from table I that a pH of 7.9 without buffer allowed 100 per cent growth. However, a buffered solution of pH 7.0 barely allowed growth, although a concentration of disodiumphosphate in that solution was only 1.2 per cent.

Since the citric acid content was present at that pH in a concentration of 0.08 per cent, it certainly played very little, if any, rôle directly. It is obvious that something besides the concentration of either the citric acid or disodiumphosphate or just the pH produced, retards growth at a pH of 7.0. It is possible that the fact that there is a tendency to have a maintained hydrogen-ion concentration in a buffered solution might explain this phenomenon.

TABLE III

Change in the pH of liquid media by Trichophyton gypseum

DURATION OF GROWTH		pH 5.0	GROWTH	pH 6.0	GROWTH	pH 7.0	GROWTH	CONTROL pH 5.8	GROWTH
days									
7	With buffer	5.6	+++	6.4	++++	6.9	+	6.9	++++
	No buffer	7.3	++++	6.8	++++	7.6	++++		
14	With buffer	5.5	+++	6.6	++++	6.9	+	7.5	++++
	No buffer	7.7	++++	7.5	++++	7.9	++++		
21	With buffer	6.5	+++	6.9	++++	6.8	+	7.6	++++
	No buffer	7.6	++++	7.9	++++	8.0	++++		

Experiment IV

A series of tubes with Sabouraud's boullion were prepared with a pH range of 5.0, 6.0, and 7.0 according to Methods I and II. These tubes were inoculated with *Trichophyton Gypseum* in the usual way. The pH was determined at intervals of 7, 14 and 21 days.

Table III summarizes the experiment. It can be seen that *Trichophyton Gypseum* may increase the pH once growth is initiated. Thus, at the end of three weeks in a solution without buffer, the effect of fungous growth was able to raise the pH of 5.0 to 7.6; with buffer it only raised the original pH of 5.0 to 6.5. Since all of the non-buffered solutions including the controls were finally all increased to about the same pH, i.e., 7.6 to 8.0, it can be concluded that that is the optimum pH range of growth for this organism. The degree of growth in all of these solutions at the end of the three weeks' period was about the same.

With buffer, when vigorous growth took place whether at an original pH of 5.0 or of 6.0, the final pH value was also about the same, i.e., 6.5 to 6.9. Thus, it was seen that the presence of the buffer tended to maintain the original pH,

and the hydrogen-ion concentration finally obtained was not the optimum required for growth. It can also be concluded, since the degree of growth at the end of three weeks for the initial pH of 6.0 was very vigorous and compared favorably with the controls, that a pH of 6.9 was also quite favorable for the development of the organism (fig. 3).

We believe that we were warranted in concluding that the presence of a buffer, when it interferes with the production of a pH value which is optimum for growth, retards the development of the colony and thus explains the less vigorous growth in the buffered solutions as compared with the non-buffered.

There was a slight change of the initial pH of 7.0 in the buffered solution to 6.9 after two weeks and to 6.8 at the end of the third week. We at first regarded this as nothing more than perhaps a factor of error in the pH reading, rather than

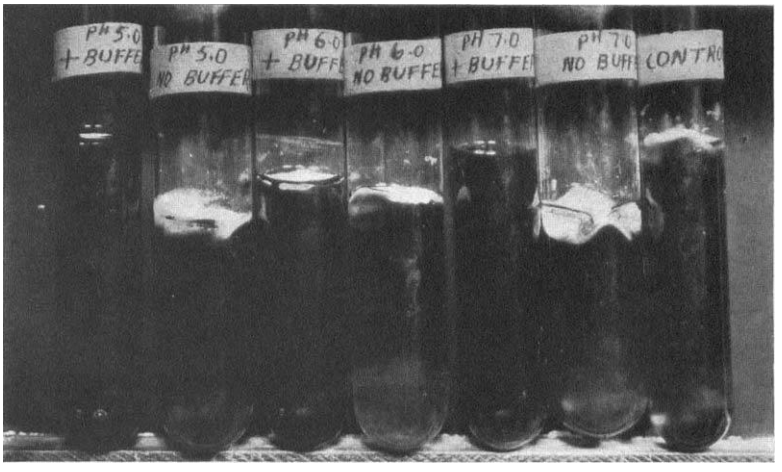


FIG. 3. GROWTH OF TRICHOPHYTON GYPSEUM ON SABOURAUD'S BOUILLON AT pH 5.0, 6.0 AND 7.0 WITH AND WITHOUT BUFFER

Note the marked retardation of growth in buffered media

actual formation of acid by the organism; especially so, since there was very little growth and that only in the depths of the media. However, a different interpretation can be made when the data in the next experiment are studied.

Experiment V

A series of tubes of Sabouraud's boullion were prepared with a pH of 3.4, 4.0, 5.0, 8.0, 9.0, and 10.0. They were buffered according to Methods I and II as usual and then inoculated with *Trichophyton Gypseum*.

Table IV summarizes the results. In the media without the buffer, up to and including a pH of 8.0, there is a change to more alkaline pH by *Trichophyton Gypseum*. As was seen from Experiment IV (table III), since an approximate pH of 8.0 was optimum for growth, there was no appreciable change in the pH

value from that of the original solution at the end of ten days, although vigorous growth took place. However, at a pH of 9.0 and 10.0, actual transformation to more acid reaction took place. The initial hydrogen-ion concentration of these solutions was brought down to 8.08 and 7.87 respectively, the higher initial pH value of 10.0 apparently serving as a stimulus for the organism to produce an acidification of the medium.

The changes in the pH values of the buffered solutions were more striking. As was seen in previous experiments, when growth took place in these solutions up to a pH of 7.0, there was a change to a more alkaline pH, just as in the non-buffered solution, but there seemed to be a slight tendency to acidify even at a pH of 7.0.

At the pH of 8.0, 9.0, and 10.0, although growth was slight and only under partial anaerobic conditions, there was a marked drop in the pH value in spite of the presence of buffers. The pH of 10.0 is brought down to 7.04; 9.0 to 7.29 and 8.0 to 7.02. Since the buffer at a pH of 8.0, 9.0, and 10.0 consisted almost entirely of disodiumphosphate in approximately 1.4 per cent concentration, it would

TABLE IV

Change in the pH of liquid media by Trichophyton gypseum

DURATION OF GROWTH		pH 3.4	Growth	pH 4.0	Growth	pH 5.0	Growth	pH 8.0	Growth	pH 9.0	Growth	pH 10.0	Growth
days													
10	With buffer	3.37	±	4.08	+	5.34	++	7.02	+	7.29	+	7.04	±
	No buffer	3.31	±	4.41	+	7.79	++++	8.17	++++	8.08	++++*	7.87	++++†

* Moderate floculate below surface.

† No floculate below surface.

seem that the presence of disodiumphosphate has a special effect on the metabolism of the *Trichophyton Gypseum*.

It similarly stimulates the production of a lower pH when it is present in sufficient concentration at a pH value of 7.0 and above. In this way, too, it may retard the growth of the fungus. Since the final pH value was not optimum for growth of the fungus, it might explain why there is a diminution of growth in the buffered solutions as compared to the non-buffered (fig. 3, table III).

Summary

In liquid Sabouraud's boullion with and without buffer, *Trichophyton Gypseum* can grow to a slight extent even at a pH of 3.4. When NaOH or HCl was used to obtain a final pH value, good growth took place in the pH range 4.4 to 10.0 (table I). When McIllvaine's buffers were added to obtain the required hydrogen ion concentration, optimum growth took place at the

initial pH of 6.0; good growth took place in the tube-buffered to 5.0, some growth at the initial pH of 4.0 and very little if any growth in the solutions which were buffered to 7.0 or above (fig. 3).

There was practically no difference in the degree of growth of *Trichophyton Gypseum* by the plate method in the whole pH range of 4.0 to 10.0 when no buffer was present. There was some retardation of the growth of *Epidermophyton Inguinale* at the pH of 4.0 of the same series. *Monilia Albicans* grew equally well at the initial pH of the plate from 4.0 to 9.0. There was some retardation at pH 10.0 (fig. 1). The addition of McIllvaine's buffer retarded the growth of the *Trichophyton Gypseum* at all pH values studied except a pH of 7.0 to 8.0. While the Epidermophyton was retarded throughout the whole series, optimum growth as compared to the controls took place at a pH of 7.0. The addition of the buffers caused some retardation in the growth of *Monilia Albicans* at all pH values (table II, fig. 2).

The inhibiting effect of the buffers on the growth of the fungi was not due to any fungicidal effects of the ingredients as such, but was due to the power of the buffered solution to maintain a pH value which is not most favorable for the growth of the fungi. In addition, disodiumphosphate has other influences on the metabolism of *Trichophyton Gypseum*. *Trichophyton Gypseum* was capable of bringing about a change to a more alkaline pH in liquid media once growth was initiated. This was true for pH 4.0 to a pH of 8.0 in media without buffer and in media with buffer from a pH of 4.0 to 7.0. At the end of three weeks it was capable of changing an initial pH of 5.0 in liquid media without buffer to a pH of 7.6. There was also a change toward the alkaline side, once growth was initiated in the media with buffers of the same pH value, but to a markedly lessened extent.

At the end of three weeks' growth in liquid media without buffer the growth of the fungus changed the pH from the initial value of 6.0 and 7.0 to 7.9 and 8.0 respectively (table III).

There was acidification of the media by *Trichophyton Gypseum* at an initial hydrogen ion concentration of 9.0 and 10.0. This tendency to acidify was even found in a buffered solution at an

initial pH of 7.0 to a slight extent. The process of acidification was apparently greatest in buffered solutions. An initial pH of 10.0 in the non-buffered solution, (i.e., when only NaOH was added) was changed to a pH of 7.89 at the end of ten days, while an initial pH of 10.0 with buffers, (i.e., sodium phosphate plus NaOH,) was brought down to a final pH of 7.04 in the same period of time (table IV).

Since at the end of ten days to three weeks the final pH with optimum growth, starting from an inital pH of 5.0 all the way up to 10.0, was finally between 7.6 to 8.0, we can conclude (1) that the optimum growth for *Trichophyton Gypseum* lies in this pH range and (2) that this organism is capable of changing the pH value towards the alkaline or acid side to bring about a pH value which is optimum for its growth.

The presence of any substance such as buffers or disodium-phosphate alone, which interferes with the final attainment of the pH necessary for optimum growth, causes a retardation in the development of the fungus. This was especially noticeable when the initial growth of the fungus took place under partially anaerobic conditions as in liquid media. It can be seen, therefore, that in preparing media of a definite pH value that is supposed to be optimal for the growth of any fungus, one must bear in mind first of all the conditions most favorable to the initiation of growth, secondly whether the materials and buffers used do not of themselves retard growth, and finally whether the character of the buffer is such that it will not interfere with the final attainment of a pH value which is optimal for the growth of the organisms in question.

THE FUNGICIDAL EFFECTS OF THE FATTY ACIDS AND THEIR SALTS

The fungicidal powers of the fatty acids from formic acid with one carbon atom to capric acid with 10 carbon atoms, as well as the unsaturated undecylenic acid, were studied on *Trichophyton Gypseum* according to the pellicle method. In a number of instances the isomers of the acids as well as their normal salts were also investigated.

Formic acid, although a weak acid as compared to hydrochloric

acid, is the strongest member of the series; acetic acid, however, is not appreciably stronger than the acids of higher molecular weight. The dissociation constants for some of the series are given below:

	K
Formic acid	0.0214
Acetic acid	0.0018
Propionic acid	0.0014
Butyric acid	0.0015
Valeric acid	0.0016
Caproic acid	0.0014
Oenanthic acid	0.00146

In each instance a series of tubes of Sabouraud's boullion was prepared with a wide range of concentrations. It has been noted in many experiments that the *Trichophyton Gypseum* was much more resistant to fungicides than *Epidermophyton Inguinale* and as a rule less resistant than *Monilia Albicans.*

The action of the individual fatty acids is discussed in some detail because it has been found that this information is not available in any single source.

1. Formic Acid: HCOOH. It is soluble in alcohol and water to infinity. The sodium salt was studied.

Pure formic acid is a colorless liquid of sharply irritating odor. It is very irritating to the skin and therefore cannot be used in certain forms of therapy. It has been used in canning as a preservative because its antiseptic power is known.

Fungicidal Power. The acid and the salt were tested only by the pellicle method on the *Trichophyton Gypseum.* A concentration of 0.03 per cent was required to inhibit growth completely. The pH of such a solution in liquid Sabouraud's medium was 4.3. It can be assumed, therefore, that only part of the inhibition of growth was due to the hydrogen-ion concentration produced (see table I). The sodium salt was appreciably weaker as it required a concentration of 0.5 per cent to totally inhibit growth.

2. Acetic Acid: $CH_3 \cdot COOH$. It is soluble in water in all proportions; also ether and alcohol. The sodium salt was studied.

Acetic acid is found in animal secretions and in sweat. Many bacteria and certain fungi have the power to split off acetic acid from organic substances.

Fungicidal Power. Acetic acid is about the same as formic acid in its fungicidal properties. It also required a concentration of 0.03 per cent to inhibit growth completely. The pH of such a solution is 4.8, well within the limits of growth for this organism as far as the hydrogen-ion concentration is concerned. The sodium salt is markedly poor as a fungicide, requiring 5 per cent to inhibit growth of the *Trichophyton Gypseum* by the pellicle method totally.

By the plate method even a 5 per cent concentration allowed 30 per cent growth of this organism. This, however, is the only salt of those studied *which seemed to be more fungicidal for Monilia Albicans than for either Trichophyton Gypseum or Epidermophyton Inguinale.* A 1 per cent concentration by the plate method inhibited the growth of Monilia much more markedly than the other two organisms.

3. Propionic Acid: $CH_3 \cdot CH_2 \cdot COOH$. It is soluble in water, alcohol and ether. Sodium, ammonium and potassium salts were studied.

No mention of this substance is made in the recent literature on sweat.

Fungicidal Powers. Propionic acid totally inhibited growth in a concentration of 0.03 per cent. This concentration gives a pH of 5.0 in Sabouraud's boullion. There was no difference in the fungistatic action of either its sodium, ammonium or potassium salts. At a pH of 5.6 it also required a concentration of 0.03 per cent to inhibit the growth of *Trichophyton Gypseum* by the pellicle method. By the plate method (table VI) 0.5 per cent was required to inhibit the growth of the same organism. It only required 0.1 per cent to inhibit totally the growth of *Epidermophyton Inguinale,* and it needed as much as 1 per cent solution to prevent growth of *Monilia Albicans.* In another paper we will discuss the variations in concentration necessary to inhibit totally growth at different hydrogen-ion concentration of this salt.

4. *Butyric Acid:* $CH_3 \cdot CH_2 \cdot CH_2 \cdot COOH$. Butyric acid and isobutyric acid, $(CH_3)_2CH \cdot COOH$ are soluble in water, alcohol and ether. The normal sodium salt was studied.

The normal acid occurs as an ester of glycerol in butter and from this source it has received its name. The free acid occurs in the feces and in perspiration. The isomeric acid occurs to a limited extent in nature. It resembles the normal acid in physical and chemical properties. Among the higher members of the series *it is almost exclusively the normal members containing an even number of carbon atoms which are found in nature.* Those with odd numbers are either not met with or are extremely rare.

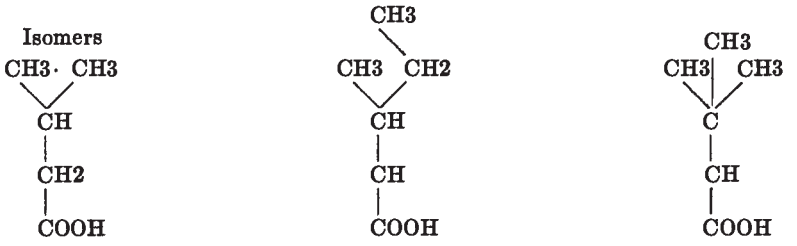
Fungicidal Powers. Butyric acid in a concentration of 0.01 per cent completely inhibits *Trichophyton Gypseum* by the pellicle method. The pH of this solution is 5.3, well within the hydrogen-ion concentration range in which this organism can grow. Iso-butyric acid required a concentration of 0.07 per cent to inhibit growth totally. This is a typical example of the reduced fungicidal powers of all the branched chain isomers studied. The sodium salt completely inhibited growth at a concentration of 0.05 per cent.

By the plate method, sodium butyrate required about a 0.25 per cent concentration to inhibit completely growth of *Trichophyton Gypseum.* It was about the same in its fungicidal powers as sodium propionate in its effect on *Epidermophyton Inguinale.* On the other hand, it required a 5 per cent solution to inhibit *Monilia Albicans.* This salt was, of those studied, the weakest fungicide for this organism.

5. Valeric Acid: $CH_3 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot COOH$. It is soluble in alcohol and ether, moderately soluble in water. Three isomers are known: iso-valeric acid, methyl-ethyl-acetic acid, and tri-methyl-acetic acid. We studied the first two isomers only.

The sodium salt of the normal acid was studied.

The normal valeric acid is found in plants but not in animal metabolism.



Iso-valeric Acid Methyl-ethyl acetic acid

Valeric acid completely inhibited growth of *Trichophyton Gypseum* in a concentration of 0.003 per cent. This is the first example of the tendency to greater fungicidal action of a fatty acid with an odd number of carbon atoms than one with an even number. Both iso-valeric acid and methyl-ethyl acetic were much less fungicidal than the normal acid. They required a concentration of 0.03 per cent to inhibit growth totally. The sodium salt required a concentration of 0.5 per cent to inhibit growth totally.

6. N. Caproic Acid: $CH_3 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot COOH$. It is soluble in alcohol and ether, slightly soluble in water.

Caproic acid is probably found in sweat. It is a colorless liquid with a sharp odor like butyric acid.

Fungicidal Powers. Caproic acid was fungistatic in concentrations of 0.009 per cent. It can thus be seen that the even-numbered carbon chain next higher in the series was less fungistatic than the valeric acid.

7. Oenanthic Acid: $CH_3(CH_2)_5 \cdot COOH$. It is slightly soluble in water, soluble in alcohol and ether.

This acid is also known as n-heptylic acid. It is a colorless liquid with a sharp odor.

Fungicidal Powers. This is another odd-numbered carbon chain acid which shows the trend for the odd number carbon chain acids to be more fungicidal than the even ones. It completely inhibited the growth of the fungi in a concentration of 0.007 per cent.

8. Caprylic Acid: $CH_3(CH_2)_6 \cdot COOH$. It is somewhat soluble in water but soluble in alcohol and ether.

It has a sharp odor and may be found in sweat.

Fungicidal Powers. Here we have a striking example of the decrease in fungicidal power of an even-numbered carbon chain compared to a previous odd-numbered chain acid. Although next higher in the series, it required a concentration of 0.03 per cent to inhibit growth completely.

9. Pelargonic Acid: $CH_3(CH_2)_7 \cdot COOH$. It is very slightly soluble in water and soluble in alcohol and ether.

This is a colorless liquid with a rather sharp acrid odor.

Fungicidal Powers. It required a concentration of 0.009 per cent to inhibit growth. This showed a marked difference in fungicidal action as compared to the previous even-numbered carbon chain. However, it is the only one of the

acids with an odd number of carbon atoms in the series above propionic which did not show a greater fungicidal power than some of the acids with an even number of carbon atoms, such as caproic and capric.

10. Capric Acid: $CH_3(CH_2)_8 \cdot COOH$. It is slightly soluble in water, and soluble in alcohol and ether.

Capric acid is a colorless liquid and probably found in sweat.

Fungicidal Powers. It required a concentration of 0.009 per cent to inhibit growth.

11. Undecylenic Acid: $CH_3(CH_2)_7 \cdot CH{=}CH \cdot COOH$. It is practically insoluble in water; soluble in alcohol.

This is a colorless liquid which is probably not found in organic material.

Fungicidal Powers. Here is a demonstration of the fungistatic action of an acid with a double bond in its chain. It required 0.005 per cent to inhibit growth totally. The sodium salt is the only one of the salts of the series studied which proved more fungicidal than its corresponding acid. Complete inhibition of growth was obtained in a concentration of 0.0009 per cent. This might be due, however, to its increased solubility.

Summary

Tables V and VI summarize a great many experiments. The fatty acids beginning with formic acid with 1 carbon atom and going up to capric acid with 10 carbon atoms as well as the unsaturated undecylenic acid, were studied for their fungicidal powers.

It can be seen from table V that with the possible exception of formic acid, the hydrogen-ion concentration resulting from the addition of sufficient of the respective acid to inhibit growth completely in liquid media was well within the pH range which would allow the *Trichophyton Gypseum* to grow.

In every instance the isomers of the fatty acids studied were much less fungicidal than the normal acids (figs. 4 and 5). As a rule, the normal salts were also less fungicidal than their corresponding acids. Sodium propionate, however, was as effective as propionic acid, while sodium undecylenate was even more effective than its acid as a fungicide. It required a concentration of 0.005 per cent of undecylenic acid to inhibit completely the growth of the fungus, while a concentration of only 0.0009 per cent of the sodium undecylenate was fungicidal. This salt was the most powerful fungicide of the series given in table V. This probably demonstrates the importance of a double bond in the chain as the reason for its greater fungicidal action.

The concentrations of fatty acids necessary completely to inhibit growth of *Trichophyton Gypseum* by the pellicle method varies from 0.03 per cent to 0.003 per cent. The most powerful fungicidal action was that of valeric acid with 5 carbon atoms. In the series above propionic acid, those fatty acids with an odd

TABLE V

Effects of fatty acids, their salts and isomers, on growth of Trichophyton gypseum (pellicle method)

SUBSTANCE STUDIED	CHEMICAL FORMULA	CONCENTRATION REQUIRED TO PREVENT GROWTH	pH
		per cent	
Formic acid	CH_2O_2	0.03	4.3
Sodium formate		0.5	5.8
Acetic acid	$C_2H_4O_2$	0.03	4.8
Sodium acetate		5.0	6.7
Propionic acid	$C_3H_6O_2$	0.03	5.0
Na propionate		0.03	5.6
Butyric acid	$C_4H_8O_2$	0.01	5.3
Sodium butyrate		0.05	5.5
Iso-butyric acid	$C_4H_8O_2$	0.07	4.8
Valeric acid	$C_5H_{10}O_2$	0.003	5.4
Sodium valerate		0.5	8.2
Iso-valeric acid	$C_5H_{10}O_2$	0.03	5.1
Methylethyl acetic acid	$C_5H_{10}O_2$	0.03	5.1
Caproic acid	$C_6H_{12}O_2$	0.009	5.5
Oenenthic acid	$C_7H_{14}O_2$	0.007	5.6
Caprylic acid	$C_8H_{16}O_2$	0.03	5.5
Pelargonic acid	$C_9H_{18}O_2$	0.009	5.7
Capric acid	$C_{10}H_{20}O_2$	0.009	5.7
Undecylenic acid	$C_{11}H_{20}O_2$	0.005	5.8
Sodium undecylenate		0.0009	5.7
Control	Sabouraud's boullion		5.8

number of carbon atoms tended to be more fungicidal than those with an even number. Pelargonic acid (9 carbon atoms) was the only one in the series studied which broke this rule, but was as powerful a fungicide as the strongest of the even numbered carbon chains, i.e., 0.009 per cent concentration was necessary to inhibit growth totally.

TABLE VI

Effect on growth of pathogenic fungi (plate method): Two weeks' growth

CONCENTRATION	SODIUM ACETATE			SODIUM PROPIONATE			SODIUM BUTYRATE			SODIUM UNDECYLENATE			CEVITAMIC ACID		
	Trichophyton gypseum	*Epidermophyton inguinale*	*Monilia albicans*	*Trichophyton gypseum*	*Epidermophyton inguinale*	*Monilia albicans*	*Trichophyton gypseum*	*Epidermophyton inguinale*	*Monilia albicans*	*Trichophyton gypseum*	*Epidermophyton inguinale*	*Monilia albicans*	*Trichophyton gypseum*	*Epidermophyton inguinale*	*Monilia albicans*
p.p.m.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*			
Control	100	100	100	100	100	100	100	100	100	100	100	100	5.4 cm.	2.7 cm.	Many small colonies
5%–50,000	20	0	10	0	0	0	0	0	0	0	0	0		Not done	Not done
1%–10,000	90	65	20	0	0	0	0	0	20	0	0	0		Agar did not harden	
													per cent	*per cent*	
0.5%–5,000	100	100	75	0	0	30	0	0	40	0	0	0	10	0	Some retardation
0.25%–2,500	100	100	100	66	0	50	65	0	40	0	0	0	50	0	No retardation
0.1%–1,000	100	100	100	90	0	100	100	40	50	0	0	0	85	85	No retardation
0.05-500	100	100	100	100	69	100	100	70	50	0	0	0	100	100	No retardation
0.025–250	100	100	100	100	100	100	100	100	50	0	0	0			
0.01–100	100	100	100	100	100	100	100	100		0	0	0			
0.005-50										0	0	0			
0.001–10										90	Alive	100			
0.0005-5										100	66	100			

0, no growth; %, as compared with control taken as 100 per cent.

FIG. 4. FUNGICIDAL ACTION OF VALERIC ACID ON TRICHOPHYTON GYPSEUM IN LIQUID MEDIUM

Fungicidal at a concentration of 0.003 per cent

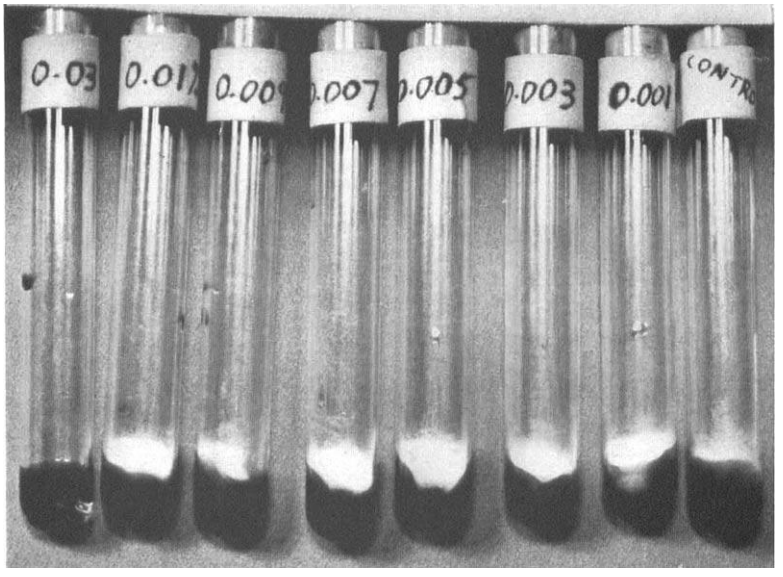


FIG. 5. FUNGICIDAL ACTION OF ISO-VALERIC ACID ON TRICHOPHYTON GYPSEUM IN LIQUID MEDIUM

Fungicidal at a concentration of 0.03 per cent

It is of interest to note that it is the branched chain isomers of the fatty acids and those with an odd number of carbon atoms which are not found in nature. The investigations indicate that there is a definite relationship between structure and fungicidal action of fatty acids.

Table VI is a summary of experiments by the plate method, which shows the effects of the salts of some of the fatty acids on the growth of *Trichophyton Gypseum*, *Epidermophyton Inguinale* and *Monilia Albicans*. As a rule, the *Monilia Albicans* was much more resistant than either the *Trichophyton Gypseum* or the *Epidermophyton Inguinale* to the action of the salts of the fatty acids, while the *Epidermophyton Inguinale* was most vulnerable.

While sodium acetate was a poor fungicide requiring over 5 per cent to inhibit totally the growth of *Trichophyton Gypseum*, it was the only salt studied which seemed to be more fungicidal for *Monilia Albicans* than for *Trichophyton Gypseum* or *Epidermophyton Inguinale.*

The marked fungicidal power of sodium undecylenate was strikingly demonstrated as compared with the salts of the other fatty acids studied. It can be seen that it inhibited growth of all three organisms completely, in a concentration of 50 parts in a million. This might be explained by the fact that it is a salt of an unsaturated fatty acid.

It can be seen from tables V and VI that *Trichophyton Gypseum* was more resistant to the action of fungicides when it was cultured under purely aerobic conditions as by the plate method than when growth was initiated under anaerobic conditions as in the pellicle method.

The effect of cevitamic acid on the growth of fungi is also given in table VI, but this will be discussed later.

THE EFFECT OF ADDITION OF VITAMINS TO CULTURE MEDIA ON THE GROWTH OF FUNGI

While we were studying the effects of various constituents of sweat on the growth of pathogenic fungi, it was observed that the addition of cevitamic acid to nutrient media, both solid and

liquid, apparently retarded the growth of certain of the pathogenic fungi. It was then decided to study the effects of the addition of vitamins A, B, and D to the same type of media on the growth of these fungi.

TABLE VII

Effect of vitamins on the growth of fungi (pellicle method)

SUBSTANCE TESTED	VITAMIN A	VITAMIN B	VITAMIN D			CEVITAMIC ACID	pH VITAMIN C SOLUTION
	Carotene in oil	Betalin S, synthetic B	Appollothron natural fish oil	Crystaline vitamin D	Propylene glycol		
per cent							
9	++++	++++	++++	0	0	0	
7				0	0	0	
5				+	+	0	
3				+++	+++	0	
1				++++	++++	0	
0.9						0	
0.7						0	
0.5						±	
0.3						+	3.8
0.1						++	4.5
0.09						+++	4.8
0.07						++++	4.9
Control	++++ Wide pellicle	++++	++++	++++	++++	++++	

++++ = Heavy growth of surface mycelium with heavy subsurface floc.
+++ = Partial covering of surface with heavy subsurface floc.
++ = Moderate subsurface growth with a small colony floating on surface.
+ = Moderate floc below surface.
± = Slight growth below surface.
0 = No growth.

Materials Used. Vitamin A: A solution of carotene in cottonseed oil (Smaco). The potency of this compound was given as 7,500 new U.S.P. units of vitamin A per gram. This is said to be the form in which pro-vitamin A is found in fruits and vegetables.

Vitamin B: (Betalin S): Synthetic vitamin B (Lilly): Each cc. is equivalent to 400 Sherman units of vitamin B_1.

Vitamin D: (Apolathron, J. B. Roerig & Co.): The natural vitamin D, each capsule (0.5 cc.) containing 25,000 units vitamin D, U.S.P. Drisdol (Winthrop

Chemical Company) crystalline vitamin D in propyline glycol is said to contain 10,000 U.S.P. units of vitamin D per gram.

Vitamin C: Crystalline vitamin C (Cebione. Cevitamic acid Merck). 25 mg. are equal to 500 international units.

Experimental Part. The effect of the addition of vitamins A, B, C, and D on the growth of fungi was compared by the pellicle method. Vitamin C was tested on solid Sabouraud's media also for its effect on *Trichophyton Gypseum*, *Epidermophyton Inguinale* and *Monilia Albicans*.

It was difficult, of course, to get an oily solution such as vitamin A and the natural vitamin D (Apolathron) to disperse through the liquid media. In table

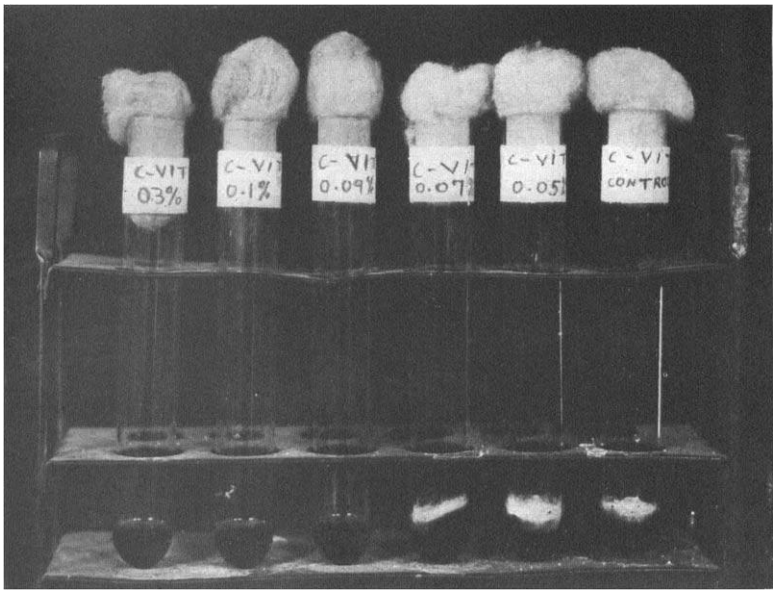


FIG. 6. FUNGICIDAL ACTION OF CEVITAMIC ACID ON TRICHOPHYTON GYPSEUM ON SABOURAUD'S BOUILLON

VII there are expressed the percentage volumes although the oily solutions formed a film on the surface of the Sabouraud's bouillon. The organisms were inoculated on the depths of the media as usual. In order to control the effect of propylene glycol on the growth of fungi this was included as one of the test substances—as can be seen from table VII. Drisdol, the crystalline vitamin D used, was dissolved in propylene glycol which was easily dispersed through the liquid Sabouraud's medium.

Results of the Experiment. Vitamins A, B, and D had no inhibiting effect on the growth of the fungi. The apparent retardation of growth by the crystalline vitamin D was due to the fact that it was dissolved in propylene glycol which itself was fungicidal in about 5 per cent concentration.

Vitamin C, however, was fungicidal for *Trichophyton Gypseum* at a concentration of about 0.5 per cent but was still fungistatic at a concentration of 0.09 per cent by the pellicle method (table VII). It can be seen from previous experiments on the hydrogen ion effect on the growth of fungi by the pellicle method that the inhibiting effect of vitamin C on the growth of *Trichophyton Gypseum* was certainly not due primarily to a hydrogen-ion effect. It can be said with justice, however, that some of the retardation of growth at a concentration of 0.3 per cent might be partly due to a hydrogen-ion effect since the culture media with that amount of vitamin C had a pH of 3.8.

A study of the effect of vitamin C on *Trichophyton Gypseum*, *Epidermophyton Inguinale* and *Monilia Albicans* by the plate method shows that the *Trichophyton Gypseum* by this method was inhibited also by a concentration of 0.5 per cent and not until there was a concentration of cevitamic acid of 0.05 per cent did a 100 per cent growth of the organism appear. *Epidermophyton Inguinale* was completely inhibited by a concentration of 0.25 per cent while even *Monilia Albicans* showed retardation of growth at a concentration of 0.5 per cent cevitamic acid.

Since we have demonstrated that all of these organisms by the plate method can grow in a pH range from 4.0 to 10.0 when hydrochloric acid and sodium hydroxide were used to adjust the pH, it seems justified to assume that both the fungicidal and fungistatic effects on these organisms were due to the cevitamic acid directly (fig. 6).

DISCUSSION

It is obvious from the experiments just cited that the differences in the results which were obtained by the various authors who reported on the optimum pH for the growth of fungi was certainly due to a great extent to the different chemicals and media used for the growth of the fungi (1, 1a, 2, 4, 5, 6, 7). In the main, however, most of the authors agree that fungi, including *Achorion Quinckeanum* (1a), *Trichophyton Gypseum* and *Epidermophyton Inguinale*, as well as *Monilia Albicans* can grow over a wide range of hydrogen-ion concentrations (4.0 to 9.0).

Several previous observers have pointed out that both *Trichophyton Gypseum* and *Epidermophyton Inguinale* can change the pH towards the alkaline side (8, 9). Mallinckrodt-Haupt (8) also stated that moulds could build acid as part of their carbohydrate metabolism. However, to our knowledge, none of the previous observers has brought out the fact that *Trichophyton Gypseum* is capable of changing the initial pH towards the alkaline or acid side in what is apparently an attempt to adjust the pH of the media to that which is optimum for its growth.

Our experiments show that it is difficult, if not impossible, to conclude anything about the optimum pH necessary for growth of any fungus by observing the degree of development of the colony or colonies at an initial pH. What can be observed without continuous electrometric pH measurements is the optimal hydrogen-ion concentration at which growth can be initiated. Once the organism begins to grow it can change the initial pH to one that is optimal for its development provided that there is nothing in the media to interfere with this change. After growth has taken place for at least 10 days to 3 weeks, the measurement of the final pH in the presence of good growth can be taken as that pH which is optimum for growth. It was seen from our experiments that *Trichophyton Gypseum*, whether it began to grow at a pH of 4.0 or 10.0, finally adjusted the hydrogen ion concentration of the liquid media to about 8.0 or a little below that figure. Such an observation then determines that pH just cited is the one optimal for the growth of *Trichophyton Gypseum*. When we used chemical substances such as McIllvaine's buffers, which prevent any attainment of a pH necessary for maximum growth, there is, of course, a retardation of development of the organism.

There is a well known rule, the rule of Traub, which states that biologic effects of a homologous series increase with the number of carbon atoms in the chain (cited by Freundlich (10)). With the fatty acid series reported in this communication, the large effects noted by Traub certainly were not present. This was especially striking in the study of the isomeric compounds. In the case of isomers according to a table given by Freundlich (10), the surface tension of water is equally affected by propyl alcohol and iso-propyl alcohol and also equally by normal and by iso-amyl alcohol.

It was seen from our experiments that the normal acids were more fungicidal than their isomers, and the acids with even numbered carbon atoms were apparently less fungicidal than those with an odd number in their chains. Also, there was no increase of fungicidal properties with the increase in the number of the carbon atoms. Thus, valeric acid with 5 carbon atoms was the

strongest fungicide of the acids studied, even though the series up to capric with 10 carbon atoms was studied. Sodium propionate, the normal salt of an acid with 3 carbon atoms was more fungicidal than sodium valerate with 5 carbon atoms. Still another example which seemed to break the rule of Traub was caprylic acid with 8 carbon atoms which was fungicidal only at a concentration of 0.03 per cent, while oenanthic acid with 7 carbon atoms was fungicidal at a concentration of 0.007 per cent.

The action of the fatty acids and their isomers, as well as the illustration about even and odd numbered carbon atoms just cited, illustrates the relationship between structure and fungicidal, i.e., fungistatic effects. It was of interest to note the influence of an acid containing a double bond on the growth of the fungi. Sodium undecylenate, which is a salt of a mono-unsaturated fatty acid, was the strongest fungicide of all these studied.

It was found that cevitamic acid has fungicidal and fungistatic properties. It can be definitely stated that these properties were not mainly due to a hydrogen-ion effect on the growth of these organisms. Since cevitamic acid is found in sweat it is of interest to speculate whether or not there is a relationship between this fact and the variations in individual resistance to the dermatotropic organisms studied.

SUMMARY AND CONCLUSIONS

1. The effects of various hydrogen ion concentrations on the growth of *Trichophyton Gypseum*, *Epidermophyton Inguinale* and *Monilia Albicans* were studied. When NaOH or HCl were used to obtain the required initial pH value, all of the organisms grew over an initial pH range of 4.0 to 10.0. The effect of hydrogen-ion concentration on *Trichophyton Gypseum* was greater in liquid than in solid media.

The addition of McIllvaine's buffers (citric acid and disodium-phosphate) had a retarding effect on the growth of all three organisms studied. Only at a pH of 6.0 in liquid media for *Trichophyton Gypseum*, and a pH of 7.0 in solid media for *Trichophyton Gypseum* and *Epidermophyton Inguinale* was optimal

growth obtained. The growth of *Monilia Albicans* was retarded at all pH values on the buffered plates as compared with the controls.

2. *Trichophyton Gypseum* was capable of producing a more alkaline pH in Sabouraud's boullion when grown on a medium with an initial pH of 4.0 to 8.0. At an initial pH of 9.0 and 10.0 it acidified the medium. There was even some acidification in buffered solutions which had an initial pH value of 7.0. The change to a more alkaline pH was less marked in the buffered solution while the change to more acid pH was more marked in the buffered solution.

At the end of 10 to 21 days the cultures of *Trichophyton Gypseum* in Sabouraud's boullion, whether initiated at a pH of 5.0 or 10.0 finally were able to bring about a change of the pH value between 7.0 and 8.0. It can be concluded, therefore, that this range of hydrogen-ion concentration was optimal for the growth of that organism.

The retardation effect of the addition of buffers on the growth of the fungi was probably due to the interference with the formation of a final pH value which was optimal for the growth of the organism.

3. The fungicidal effects of the fatty acid series beginning with formic acid with one carbon atom and concluding with capric acid with 10 carbon atoms, as well as the unsaturated undecylenic acid, were studied. A number of the sodium salts of the normal acids and some iso-acids were also investigated for their fungicidal powers.

Valeric acid with 5 carbon atoms in its chain seems to have had the greatest fungicidal properties of all the fatty acids studied. It was fungicidal in a concentration of 0.003 per cent. There was no discernible relationship between the length of the carbon chain and the fungicidal properties of the fatty acids in the series studied. It seemed, however, that acids with an odd number of carbon atoms were more fungicidal than those with even numbers. This conclusion applies to the acids higher in the series than propionic.

The branched chain isomers studied were less fungicidal than

their corresponding normal acids. With the exception of sodium propionate and sodium undecylenate, the sodium salts of the normal acids were also less fungicidal than their corresponding acids. Sodium undecylenate was the most powerful fungicide of all the substances studied. It completely inhibited growth of *Trichophyton Gypseum* in liquid Sabouraud's medium at a concentration of 0.0009 per cent. This demonstrates an increased fungicidal effect perhaps due to the presence of a double bond in the chain.

The fungicidal and fungistatic effects of the fatty acids and their salts were not due to the hydrogen-ion concentration produced by their addition to the media studied.

4. The additions of vitamins A, B, C, and D to culture media were investigated for their effects on the growth of *Trichophyton Gypseum*, *Epidermophyton Inguinale* and *Monilia Albicans*. Vitamins A, B, and D did not affect the growth of the fungi. Cevitamic acid, however, has definite fungicidal and fungistatic properties which are not due primarily to the changes in the hydrogen ion concentration produced by the addition of this substance to the media.

(This work was done with the technical assistance of Edward Weissbard.)

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DISCUSSION

DR. JOSEPH V. KLAUDER, *Philadelphia, Pa.:* Some years ago, it was pointed out that the contents of the vesicle of tinea lesions uniformly reacted to litmus paper on the alkaline side, and therefore that acid reacting medicaments would be indicated. In conformance with that, acetic acid was recommended and also boric acid powder. That is apparently a fallacy from Dr. Peck's work. I would like to ask Dr. Peck if his studies would lead to any practical application in the therapeusis of tinea.

DR. J. GARDNER HOPKINS, *New York City:* I would like to congratulate Dr. Peck on this very interesting presentation. It seemed to me that it is an exceedingly good omen for our Society to have our program begin with a paper of this character.

DR. MARION B. SULZBERGER, *New York City:* I second Dr. Hopkins' congratulations. I think these studies are of fundamental importance and will perhaps lead to practical applications in the clinical investigation and in the treatment of fungous infections. I would like to ask Dr. Peck to explain the seeming contradiction between the clinical observation that fungous diseases often appear in precisely those areas where most perspiration gathers, and the fact that perspiration often contains many of the fungicidal ingredients he has demonstrated. Why, if the sweat contains these fungicidal fatty acids and cevitamic acid, do the fungi tend to produce clinical disease in intertriginous sites?

DR. HERMAN SHARLIT, *New York City:* Standard preparations of liquid or solid media for the growth of micro-organisms are made as they are because they have been shown to afford optimum conditions of growth. I take it that Dr. Peck has adulterated such media by the addition of buffer solutions and demonstrated that such adulteration destroyed optimum growth conditions. It has always been known that the pH of the media was an essential feature in the specifications for optimum mixtures and it could be assumed safely that any adulteration calculated to upset this feature of hydrion concentration would effectively operate to produce inferior media. Dr. Peck's experiment has verified this assumption.

The contamination of standard media with chemicals to prove their fungicidal or fungistatic values has persistently failed to lead to materially helpful suggestions for aid in the cure of tinea. There are distinct objections to the method of directly contaminating media with chemicals for studies in fungicidity. It was for this reason that several years ago I undertook to study and introduce a new technic for the study of these problems in the laboratory. ("A Search for a

New Method for the Determination of the Fungicidal Action of Chemicals." Archiv. Derm. and Syph., April, 1932. And "The 'Membrane Method' for Determining the Fungicidal Action of Chemicals." Archiv. Derm. and Syph., Feb., 1935.) This "Membrane Method" technic succeeded at least in establishing in the laboratory the fungicidal and fungistatic values of boric and salicylic acids, a fact accepted clinically but not established by the usual practice of media contamination experiments. I am sorry that Dr. Peck did not use the "Membrane Method" in these studies of his.

DR. JOHN H. STOKES, *Philadelphia:* I would like to direct attention to one point which is to me significant for future study, namely that an organism of whatever type prepares the ground about it by producing its own optimum pH and protecting itself from invasion by other bacteria. I have seen attempts made to modify the behavior of cultures on the human toe by the introduction of other organisms which would supposedly change the biological situation for the fungus and produce an antagonistic environment. I have witnessed their cultural suggestiveness and their flat failure on the foot. I am impressed by the fact that we have always underrated the ability of the microörganism to meet conditions as it finds them. The fact that the microörganism is singularly intelligent is seldom reckoned with in work of this sort, and the attempt to transfer a test tube situation to the far more highly complex biological medium of the human skin has an a priori expectation of failure. That is why there exist such a multitude of fungicidal and fungistatic preparations put out by drug houses, et cetera, all of them with a certain amount of success. They fail to reckon with the fact that they were tested out and introduced in the first place because they seemed to work on culture media. The organism on a culture medium is meeting an entirely different situation from the growth situation on the human skin. Substances which are fungicidal or fungistatic in culture will not have corresponding actions on the skin. That consideration if taken seriously would take off the market a great many preparations which have nothing to recommend them except their test tube eligibility. I think therefore there is much to look forward to in that Dr. Peck is going to study the biological foundation for the ability of the fungus to meet the conditions of its terrain and reproduce its own biological environment. That, is very important, how the organism makes the skin a place on which it can grow even under what may be adverse and certainly highly complex conditions.

DR. FRED D. WEIDMAN, *Philadelphia, Pa.:* The impression is widespread that an acid medium is necessary for the growth of fungi. That is not the case. So far as mere growth is concerned, our pathogenic fungi do very well on a wide variety of media. The reason why the idea of a low pH has gained emphasis in mycological culture work in general is because on such a medium the peculiar and distinguishing characteristics of the fungus are thereby brought out to the best advantage. The problem of mycology today is very largely that of identification of species, and in order that that may come to pass, it is necessary that the special characteristics of the fungus should be developed; this is an entirely different matter from the question of mere luxuriance of growth. Dr. Peck has indicated that as luxuriant a growth was produced on a pH of 7.6 as with the low pH, and regardless of whether there were buffers present or not.

The second point I want to touch upon is that of inhibition of growth of bacteria in the immediate neighborhood of the fungus culture. I think that depends on the bacterial species concerned. A good many years ago Chambers found *B. subtilis* on culture would inhibit the growth of fungi and he was led to attempt therapy with that agent by incorporating it in an ointment. (A Fungistatic Strain of Bacillus Subtilis Isolated from Normal Toes. Weidman, F. D. and Chambers, S. D., Arch. Dermat. & Syph., **18:** 568 (Oct.) 1928.) It was encouraging but not final. Personally I feel that the zone of inhibition of growth around the fungus is not one that holds good for all species of bacteria, and that upon testing, certain ones can be classified which are not influenced and others that are influenced.

Dr. Samuel M. Peck: I wish to thank the discussers. We were very careful to state in our introduction that we did not wish to create the impression that we were introducing any astonishingly new and effective fungicides. The fatty acids were very interesting to us because some of them are found in sweat and thus their presence might explain the fungicidal action of sweat, if such a fact were true. In addition, by using some of these fatty acids which are found in sweat as therapeutic agents, we are introducing a more physiological method of treatment. In another communication which I am reading before the American Dermatological Association, the practical aspects of this problem are presented. Patients with fungicidal infections are treated with these fatty acids both singly and in combinations.

Unfortunately, the literature reveals many errors in the conception of the function of perspiration. Most authors believe that increased perspiration prepares the ground for fungicidal infection. Just the converse is true. Wetness on the surface of the skin is produced by two different mechanisms. One is the so-called insensible perspiration which contains practically nothing but water and a little salt. This is not produced by the sweat glands; and this fluid, present between the toes and in areas where maceration occurs, certainly provides ideal conditions for fungous infection. Areas which are bathed by true sweat, provided it is not too dilute, are actually protected against parasitic invasion.

The application of the use of fatty acids has as yet not been accurately developed. An ideal method of treatment from a purely theoretical point of view would be ingestion of foods and even some of the fatty acids themselves, so that the content of substances like propionic acid, butyric, lactic acid, and vitamin C, are increased in the sweat so that we have a natural protective mantle.

In answer to the question of Dr. Downing regarding possible spontaneous changes of pH we cited the pH values as accurately as we could. We can say fairly confidently that the figures as given in the first part of our paper indicate the trend, at least as far as in the change in the pH value by the fungi is concerned.